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PROXIMATE COMPOSITION AND BIOCHEMICAL CONSTITUENTS IN DIFFERENT STAGES OF *PARKIA ROXBURGHII* PODS UNDER DIFFERENT AGRO-CLIMATIC ZONES OF MANIPUR

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ABSTRACT

Different stages of Parkia roxburghii pods grown in different agro-climatic zones were analyzed for their biochemical composition. Higher content of vitamin C was recorded in the tender stage of the pod in all the agro-climatic zones, highest being recorded in the Temperate sub-alpine zone (60.8mg/100g) followed by Sub-tropical hill zone and Sub-tropical plain zone. Highest protein content was recorded in Temperate sub-alpine zone (19.68%), while the minimum content being found in Mild-tropical hill zone (15.5%). Protein content in Parkia pods differed significantly (P<0.01) in different stages and in different agro-climatic zones. Oil content in all the agro-climatic zones increased gradually from tender stage to the mature stage of the pod. However, no significant difference in oil content was observed among the agro-climatic zones. Crude fiber differed significantly in different zones and different stages of the pod (P<0.01). Higher range in crude fiber in the pods was recorded in Mild-tropical hill zone (12-23%), while the minimum range being found in Temperate sub-alpine zone (7.85-15.80%). Crude carbohydrates in the pods ranged from 58.02 to 69.04%, while it ranged from 48.61-50.0% in the seeds. Calorific value was higher in the seeds ranging from 437.25-448.90 kJ/100g than the pods. However, no significant differences were observed in crude fat, crude carbohydrate, total ash and gross energy (GE) in different agro-climatic zones. Values of Free amino acids, starch, total soluble phenols, orthodihydric phenols and bound phenols were not significantly differed among the four agro-climatic zones, however, TSAZ recorded significantly (P>0.01) higher values of total soluble sugars (4186.57mg/100g) and tannins (2909.20mg/100g) from the rest of the zones.

KEYWORDS: P. Roxburghii, Biochemicals, Proximate Composition, Gross Energy, Phenolics

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INTRODUCTION

The genus Parkia is known to have 40 species widely distributed in tropical parts of the world, out of which 3 species occur in India (Willis, 1982). *Parkia roxburghii* G. Don (common name: tree bean) is one the most common species widely grown here for its various edible parts of the plant. It is a much valued vegetable in Manipur, some northeastern states and other southeastern Asian countries. Due to its wide distribution, it has also been accessioned under different names (Synonyms) *Parkia javanica* (Lam.) Merr. and *Parkia timoriana* (DC.) Merr. (Anon, 1981). In Manipur, people generally belief that *P. roxburghii* grown in colder areas have superior palatability than the other regions. Keeping the above point in mind, the present experiment was conducted so as to have some idea about the biochemical composition of the pods in different agro-climatic regions of the state.

MATERIALS AND METHODS

Proximate Analysis

According to research review committee report (Anon, 1984) Manipur falls under four agro-climatic regions- Temperate sub-alpine zone (TSAZ), Sub-tropical hill zone (STHZ), mild tropical hill zone (MTHZ) and Sub-tropical plain zone (STPZ). Different stages of *Parkia roxburghii* pods were collected from 3 main marketplaces fall within each zone (Fig 1). They were classified into 4 groups based on the mean thickness of the pod measured at the site of seeds as- Tender (6mm and below); Immature (6.1 to 10 mm), Mature (more than 10 mm) and seeds (when the pulp could not be used further). The pods after removing the outer green peel and other non consumables were cut into two sizes- one portion, approximately 2-3mm in size was used for the determination of moisture, acidity and ascorbic acid as per procedures described by Jayaraman (1988). The other portion, about 5-6 mm in size was dried in an oven at 70^{o}C for 48 hours. The samples were then ground to a fine powder by using a Remi grinder and sieved (1mm). The powder samples were stored in desiccators until analysis. Crude fat (soxhlet extraction method), crude fibre, crude protein (kjeldahl method using Kel-Plus Supra LX autoanalyser) and total ash were carried out as per procedures described by Chopra and Kanwar (1980). Crude carbohydrate content and gross energy were estimated following the methods of Osborne and Voogt (1978) and Müller and Tobin (1980).

Biochemical Analysis

100mg powdered sample was extracted with a small volume of 80% ethanol in a pestle and mortar for three times. The extracts were pooled and centrifuged for 5 minutes. The volume of the supernatant was made up to 20 ml with 80% ethanol and transferred into batches of 5 ml cryo vials and stored at -20^{o}C until use. The residue left in the tube was dried in an oven at 50^{o}C for 24 hrs and used for the estimation of starch following the method of Morris (1948). 1 ml of the extract from the cryo vial was evaporated to almost dryness and dissolved in 5 ml distilled water. The extract was used for the determination of total soluble sugar, total free amino acids, total soluble phenols, and tannins following the procedures described by Sadasivam and Manikam (1992). Orthodihydric phenols and bound phenols were determined as per the methods described by Mahadevan and Sridhar (1986) and Thimmaiah (2006).

Statistical Analysis

All measurements were carried out in triplicate for each of the sample. Data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS for windows version 10.0.

RESULTS AND DISCUSSIONS

Moisture, acidity and ascorbic acid content were indicated in Table 1. The data on acidity showed a clear decreasing trend as the pod matures. It may be due to the reason that the organic acids are converted to sugars or ultimately to starch because it is the predominant form of storage food in plants. Higher content of vitamin C is recorded in the tender stage of the pod in all the agro-climatic zones, highest (60.8%) being recorded in the TSAZ followed by STHZ and STPZ. Difference in vitamin C and moisture with respect to genotypes and environment are also reported by some workers. Seeds contain lesser quantity of ascorbic acid than the mature pods thereby showing majority of the ascorbic acid is concentrated in the pods. It was also observed in other legumes (Bhagya, et al., 2006)

Data in Table 2 indicated proximate composition of the pods in different agro-climatic zones. Among the pods, higher percentage of protein was recorded in the tender stage in all the agro-climatic regions ranging from (15.5%-

19.68%). The protein content slightly goes down in the immature stage but increases again in the mature stage of the pod while maximum content being recorded in the seeds ranging from 26.23-29.5%. Such variations in protein content with the developmental stages of the pod were also observed in *Canavalia maritime* (Horace & Revo, 1985). Proteins of Parkia pods are comparable or more than the range of many wild legumes like *Atylosia scarbacoides* (17.3%), *Erythrina indica* (21.5%), *Neonotonia wightii* (15.1%), *Rhynchosia filipes* (169%), *Canavalia cathartica* (21.7%) (Arinathan et al., 2003; Bhagya et al., 2006) and edible legumes- *Cajanus cajan* (19.4%), *Cicer arietenum* (20.7%), *Vigna trilobata* (20.2%) and *V. unguiculata* (15.9%) (Jambunathan & Singh, 1980). Protein content in the Parkia pods differed significantly (P<0.01) in different stages and in different agro-climatic zones. Highest protein content was recorded in TSAZ (19.68%), while the minimum content being found in MTHZ (15.5%). Protein yields of plants are reported to vary widely with different species, different extraction methods, different stages of maturity and soil conditions. The considerable variation in protein may partly be attributed to variation in genetic make up of the genotypes studied. In addition, variation in environmental factors (soil, temperature etc.) of the study sites may also contribute for the variation in the protein content of the genotypes (Alamerew, 2003). Oil content in *P. roxburghii* seeds (19.05-20.0) was significantly higher than most of the wild edible legumes as well as common edible legumes. Majority of the edible legumes contain oil in the range of 0.5 to 5.6% except in soybean and groundnut, where it contains 19.5 and 43.4%, respectively (Chatterjee & Bhattacharya, 1986). Among the lesser known legumes, winged bean reported to contain 17.7% fat, while *Mucana gigantean* contains 5.89% (Rajara & Janardhan, 1971). The oil content in all the agro-climatic zones increased gradually from tender stage to the mature stage of the pod. However, no significant differences in oil content observed among the agro-climatic zones.

Crude fibre differed significantly in different zones and different stages of the pod (P<0.01). Higher range in crude fibre in the pods was recorded in MTHZ (12-23%), while the minimum range being found in TSAZ (7.85-15.80%), whereas fibre in the seeds ranged from 7.90-10.10%. Fibre content in wild legume seeds ranges from 2.4 to12.8% (Watson, 1977), other common legume seeds ranges from 2.7 to 8.0% except in *Lathyrus sativus* where the fibre content is 15% (Suchita, 1990). Fibre content in Parkia seeds was a little higher than most of the legumes. It may partly be due to the inclusion of seed coat (edible) during sample preparation. These results are in agreement with the reports of Balogun and Fetuga, (1986) who reported fairly high levels of crude fiber in 15 wild leguminous seeds. High crude fiber could effectively trap and protect a greater proportion of their nutrients (proteins and carbohydrates) from hydrolytic breakdown resulting in lowered digestibility and therefore reduce the extent to which their end products of digestion could be utilized Southgate (1973).

Crude carbohydrates in the pods ranged from 58.02 to 69.04%, which was lowered than the seeds (48.61-50.0%) indicating that oil is the major storage compound in the seeds by containing up to 20%. Subsequently, the calorific value was higher

Table 1: Moisture, Acidity and Ascorbic acid Content of *P. Roxburghii* in Fresh Weight in different Agro-Climatic Regions under Various Developmental Stages of the Pod (Mean±S. Em; n=6)

Consti-Tuents	Stages of the Pod	Agro-Climatic Zones				Mean (Zones)±S. Em.	±S.Ed.		C.D. at 5%		C.D. at 1%	
		TSAZ	STHZ	MTHZ	STPZ		Stage	Zone	Stage	Zones	Stage	Zones
Moisture (%)	Tender Immature Mature Seeds	82.70±1.37 75.0±0.95 69.5±1.10 60.10±1.98	80.50±0.93 76.0±1.01 73.0±1.21 58.20±1.74	80.20±0.92 77.90±1.09 74.30±1.33 59.20±1.45	84.30±1.50 78.50±1.13 76.10±1.25 59.90±1.73	81.93±0.97 76.85±0.81 73.23±1.39 59.35±0.50	1.14	1.14	2.58	NS	3.71	-
	Mean (Stage) ±S.Em.	71.83±3.53	71.93±3.01	72.90±2.99	74.70±3.23							
Acidity (%)	Tender Immature Mature Seeds	0.29±0.01 0.28±0.01 0.27±0.01 0.24±0.01	0.30±0.01 0.30±0.01 0.26±0.01 0.25±0.02	0.30±0.01 0.30±0.01 0.29±0.01 0.2±0.03	0.28±0.01 0.26±0.01 0.26±0.01 0.24±0.02	0.29±0.01 0.29±0.01 0.27±0.01 0.23±0.01	0.01	0.01	0.03	NS	0.04	-
	Mean (Stage) ±S.Em	0.27±0.01	0.28±0.01	0.27±0.02	0.26±0.01							
Vitamin C mg/100g	Tender Immature Mature Seeds	60.80±3.23 33.20±2.91 40.80±2.25 34.0±1.15	40.00±2.38 19.60±1.69 29.20±2.82 26.4±1.45	38.00±2.57 19.20±1.50 24.40±1.48 18.0±1.47	40.80±2.02 25.60±2.35 28.00±2.64 24.4±1.45	44.90±5.33 24.40±3.28 30.60±3.55 25.70±3.96	2.08	2.08	4.70	4.70	6.75	6.75
	Mean (Stage) ±S.Em	42.20±6.53	28.80±4.96	24.9±4.44	29.70±3.68							

Note: NS= Non-significant; TSAZ=Temperate sub-alpine zone, STPZ=Sub-tropical plain zone; STHZ= Sub-tropical hill zone; MTHZ=Mild tropical hill zone

Table 2: Proximate Composition of *P. Roxburghii* G. Don in different Maturity Stages of the Pod under different Agro-Climatic Zones (Mean±S. Em; n= 6)

Consti-Tuents (%)	Stages of the Pod	Agro-Climatic Zones				S.Ed±		C.D. at 5%		C.D. at 1%	
		TSAZ	STHZ	MTHZ	STPZ	Stage	Zones	Stage	Zones	Stage	Zones
Crude Protein	Tender Immature Mature Seeds	19.68±1.09 15.31±0.37 17.93±0.54 29.5±0.91	16.75±0.53 13.56±0.40 15.75±0.45 27.71±1.01	15.5±0.41 12.25±0.58 13.13±0.33 26.23±0.89	18.12±0.46 13.84±0.49 16.10±0.46 28.07±0.89	0.30	0.30	0.67	0.67	0.96	0.96
Crude Lipd	Tender Immature Mature Seeds	0.25±0.01 (0.87±0.01) 0.60±0.02 (1.05±0.03) 1.30±0.05 (1.34±0.04) 19.30±0.44 (4.45±0.21)	0.21±0.01 (0.84±0.04) 0.44±0.03 (0.97±0.03) 0.98±0.06 (1.22±0.07) 19.30±0.31 (4.45±0.31)	0.22±0.01 (0.85±0.03) 0.36±0.02 (0.93±0.04) 0.93±0.07 (1.20±0.06) 19.05±0.34 (4.42±0.17)	0.25±0.01 (0.87±0.03) 0.43±0.01 (0.96±0.04) 0.98±0.06 (1.22±0.06) 20.0±0.46 (4.43±0.16)	0.16	0.16	0.35	NS	0.50	-
Crude Fibre	Tender Immature Mature Seeds	7.85±0.31 13.80±0.34 15.80±0.62 7.90±0.42	10.00±0.47 14.40±0.50 18.20±0.58 9.30±0.44	12.00±0.42 17.00±0.64 23.00±0.65 10.10±0.43	10.80±0.65 14.30±0.45 20.10±0.70 8.80±0.46	0.74	0.74	1.68	1.68	2.42	2.42
Crude Carbohydrates	Tender Immature Mature Seeds	67.72±1.63 66.29±1.81 60.57±1.44 50.00±2.35	69.04±2.10 67.10±1.33 60.27±0.96 49.50±0.79	68.18±1.81 66.69±1.62 58.19±0.81 49.95±0.85	66.33±1.83 66.83±1.55 58.02±0.85 48.61±0.84	0.56	0.56	1.27	NS	1.82	-
Ash	Tender Immature Mature Seeds	4.50±0.12 4.00±0.08 4.40±0.18 4.00±0.15	4.00±0.12 4.50±0.11 4.80±0.16 4.00±0.11	4.10±0.13 3.70±0.22 4.75±0.16 4.40±0.13	4.50±0.14 4.60±0.10 4.80±0.18 4.00±0.10	0.21	0.21	NS	NS	-	-
Gross Energy KJ/100g	Tender Immature Mature Seeds	430.57±6.64 331.80±5.71 325.70±5.21 448.90±4.88	345.05±4.90 326.60±5.40 312.90±6.57 443.30±6.51	336.70±4.81 319.0±5.36 293.65±4.64 437.25±7.07	340.05±3.94 326.55±6.67 305.30±4.66 448.80±4.89	14.22	14.22	32.17	NS	46.21	-

Figures in bracket indicate transformed values [Transformed value=√(original value+0.5)]

In the seeds ranging from 437.25 - 448.90 kJ/100g. These results were in agreement with the reports of Seena et al., (2005). However, in Pakia pods, no significant differences were observed in case of crude fat, crude carbohydrate, total

ash and gross energy (GE) in different agro-climatic zones. This may be due to the fact that the data shown here are the pooled values of six genotypes representing one agro-climatic zone.

Table 3 indicated free amino acids (FAA), total soluble sugar (TSS) and starch content in different agro-climatic zones. FAA and starch values were not significantly differed among the four zones, while TSAZ recorded significantly (P>0.01) higher values of TSS (4186.57mg/100g) than the other three zones. Soluble sugars are well known to lower the freezing point and thus possible resistance to low temperature in the cold climates (Nautiyal, 1983). Russel, (1940) reported accumulation of soluble carbohydrates that results in higher osmotic value in plants growing in alpine and sub alpine areas, thereby increasing cold tolerance. TSAZ, being the highest (altitude) agro-climatic zone among the four regions, significantly higher values of TSS are in agreement with the above reports.

Data in Table 4 indicated the phenolic substances in the seeds in different agro-climatic zones. Total soluble phenols (TSP), orthodihydric phenols (ODHP) and bound phenols (BP) did not show any significant differences among the agro-climatic zones. Tannins were the only phenolic substance found to be significantly different among the agro-climatic zones (P>0.01), the highest being recorded in TSAZ (2909.20mg/100g). Though, presence of high amounts of tannin interferes with the digestive system (Savelkoul, et al., 1992), presence of tannin in food sometimes gives body and fullness of flavor to the food. The greatest difference in composition between cider apples and culinary apples is in the tannin content (Meyer, 1987). Naturally high tannin content is desirable for slight astringency (Valier, 1951).

CONCLUSIONS

Different pods collected from different agro-climatic zones showed no significant differences in case of crude oil, crude carbohydrate, total mineral content, total gross energy, FAA, starch, TSP, ODHP and BP. However, significantly higher values in case of vitamin C, proteins, TSS and Tannins were observed in TSAZ. As tannins are related with the flavor of foods, higher tannin content in the pods of TSAZ coupled with higher amounts of TSS and lesser values of crude fiber may enhance the palatability of the pods from this region.

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APPENDIX

Table 3: Free Amino Acid, Soluble Sugar and Starch Content in the Seeds of *Parkia Roxburghii* in different Agro-Climatic Zones (Mean ± S.Em; n=6). Figures in Parenthesis Indicate Ranges (mg/100g)

Constituents	Agro-climatic Zones				S. Ed±	C.D.	
	TSAZ	STHZ	MTHZ	STPZ		5%	1%
Free Amino acids	4698.83±188.32 (4231.0-5385.0)	3878.17±319.50 (2500.0-4615.0)	3378.33±275.56 (2231.0-4308.0)	4237.17±360.95 (3077.0-5192.0)	513.36	NS	-
Total Soluble Sugar	4186.57^{a}±909.90 (952.4-6442.6)	1980.40^{b}±386.21 (874.0-3566.8)	1834.77^{b}±613.56 (717.2-4874.0)	1972.70^{b}±691.86 (1109.2-5449.2)	603.99	1287.10	1779.95
Starch	2047.17±212.62 (1206.5-2667.6)	1617.68±345.31 (1016.0-2393.5)	1693.67±138.14 (1220.7-1980.7)	1606.95±121.68 (1192.4-1982.9)	264.78	NS	-

Note: TSAZ=Temperate sub-alpine zone; STPZ=Sub-tropical plain zone; MTHZ=Mild tropical hill zone and STHZ= Sub-tropical hill zone. Different letters with different colors in a row indicate significant at 1% level.

Table 4: Phenolics in the Seeds of *Parkia Roxburghii* in different Agro-Climatic Zones (Mean ± S.Em; n=6)

Phenolics	Agro-climatic Zones				Mean (Zones)	C.D$_{.05}$	C.D$_{.01}$
	TSAZ	STHZ	MTHZ	STPZ			
Total Soluble Phenols	527.80±49.34 (375.6-697.8)	494.00±57.13 (350.4-677.4)	464.57±45.68 (394.4-683.6)	460.07±76.57 (381.4-593.6)	486.61±15.21	NS	-
Orthodihydric phenols	229.85±3.82 (219.7-241.4)	216.88±4.14 (206.8-232.8)	226.27±3.31 (211.1-232.8)	224.82±6.93 (206.8-254.4)	224.46±	NS	-
Bound Phenols	137.33±18.41 (92.0-208)	155.67±15.08 (110.0-195.0)	185.37±11.21 (155.0-225.0)	138.50± 23.14 (74.0-210.0)	154.22±13.63	NS	-
Tannins	2909.20^{a}±260.07 (2157.4-3848.2)	1684.93^{b}±209.87 (1307.0-2595.6)	2131.37^{ab}±142.53 (1487.4-2518.2)	2393.73^{ab}±138.23 (1848.2-2874.0)	2279.81±309.30	658.81	911.08
Mean (Phenolics)	951.05±460.41	637.87±264.61	751.90±326.22	804.28±373.78			

Note: Figures in parenthesis indicate ranges (mg/100g). Different letters in a row indicate significant at 5% level while with different colors indicate significance at 1% level. TSAZ=Temperate sub-alpine zone; STHZ= Sub-tropical hill zone; MTHZ=Mild tropical hill zone and STPZ=Sub-tropical plain zone.